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CONTENTS/SUMMARIES

Summary: Frankia strains are N_2-fixing actinomycetes whose isolation and cultivation were first reported in 1978. They induce N_2-fixing root nodules on diverse nonleguminous (actinorhizal) plants that are important in ecological successions and in land reclamation and remediation. The genus Frankia encompasses a diverse group of soil actinomycetes that have in common the formation of multilocular sporangia, filamentous growth, and nitrogenase-containing vesicles enveloped in multilaminated lipid envelopes. The relatively constant morphology of vesicles in culture is modified by plant interactions in symbiosis to give a diverse array of vesicle shapes. Recent studies of the genetics and molecular genetics of these organisms have begun to provide new insights into higher-plant–bacterium interactions that lead to productive N_2-fixing symbioses. Sufficient information about the relationship of Frankia strains to other bacteria, and to each other, is now available to warrant the creation of some species based on phenotypic and genetic criteria.

Summary: Extracellular solute-binding proteins of bacteria serve as chemoreceptors, recognition constituents of transport systems, and initiators of signal transduction pathways. Over 50 sequenced periplasmic solute-binding proteins of gram-negative bacteria and homologous extracytoplasmic lipoproteins of gram-positive bacteria have been analyzed for sequence similarities, and their degrees of relatedness have been determined. Some of these proteins are homologous to cytoplasmic transcriptional regulatory proteins of bacteria; however, with the sole exception of the vitamin B_{12}-binding protein of Escherichia coli, which is homologous to human glutathione peroxidase, they are not demonstrably homologous to any of the several thousand sequenced eukaryotic proteins. Most of these proteins fall into eight distinct clusters as follows. Cluster 1 solute-binding proteins are specific for malto-oligosaccharides, multiple oligosaccharides, glycerol 3-phosphate, and iron. Cluster 2 proteins are specific for galactose, ribose, arabinose, and multiple monosaccharides, and they are homologous to a number of transcriptional regulatory proteins including the lactose, galactose, and fructose repressors of E. coli. Cluster 3 proteins are specific for histidine, lysine-arginine-ornithine, glutamine, octopine, nopaline, and basic amino acids. Cluster 4 proteins are specific for leucine and leucine-isoleucine-valine, and they are homologous to the aliphatic amidase transcriptional repressor, AmiC, of Pseudomonas aeruginosa. Cluster 5 proteins are specific for dipeptides and oligopeptides as

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well as nickel. Cluster 6 proteins are specific for sulfate, thiosulfate, and possibly phosphate. Cluster 7 proteins are specific for dicarboxylates and tricarboxylates, but these two proteins exhibit insufficient sequence similarity to establish homology. Finally, cluster 8 proteins are specific for iron complexes and possibly vitamin B_{12}. Members of each cluster of binding proteins exhibit greater sequence conservation in their N-terminal domains than in their C-terminal domains. Signature sequences for these eight protein families are presented. The results reveal that binding proteins specific for the same solute from different bacteria are generally more closely related to each other than are binding proteins specific for different solutes from the same organism, although exceptions exist. They also suggest that a requirement for high-affinity solute binding imposes severe structural constraints on a protein. The occurrence of two distinct classes of bacterial cytoplasmic repressor proteins which are homologous to two different clusters of periplasmic binding proteins suggests that the gene-splicing events which allowed functional conversion of these proteins with retention of domain structure have occurred repeatedly during evolutionary history. On the basis of results reported, as well as previously published sequence and three-dimensional analyses, it is tentatively proposed that many of the periplasmic solute-binding proteins, together with the homologous cytoplasmic DNA-binding proteins, make up a single superfamily. The divergence of cytoplasmic repressor proteins from periplasmic receptor proteins must have occurred after the proposed duplication and divergence events which gave rise to the eight major families of external receptors characterized in this report.

Summary: A variety of pathogenic bacteria produce phospholipases C, and since the discovery in 1944 that a bacterial toxin (Clostridium perfringens alpha-toxin) possessed an enzymatic activity, there has been considerable interest in this class of proteins. Initial speculation that all phospholipases C would have lethal properties has not been substantiated. Most of the characterized enzymes fall into one of four groups of structurally related proteins: the zinc-metallophospholipases C, the sphingomyelinases, the phosphatidylinositol-hydrolyzing enzymes, and the pseudomonad phospholipases C. The zinc-metallophospholipases C have been most intensively studied, and lethal toxins within this group possess an additional domain. The toxic phospholipases C can interact with eukaryotic cell membranes and hydrolyze phosphatidylcholine and sphingomyelin, leading to cell lysis. However, measurement of the cytolytic potential or lethality of phospholipases C may not accurately indicate their roles in the pathogenesis of disease. Subcytolytic concentrations of phospholipase C can perturb host cells by activating the arachidonic acid cascade or protein kinase C. Nonlethal phospholipases C, such as the Listeria monocytogenes PLC-A, appear to enhance the release of the organism from the host cell phagosome. Since some phospholipases C play important roles in the pathogenesis of disease, they could form components of vaccines. A greater understanding of the modes of action and structure-function relationships of phospholipases C will facilitate the interpretation of studies in which these enzymes are used as membrane probes and will enhance the use of these proteins as models for eukaryotic phospholipases C.

Summary: A role for Ca^{2+} in the tip growth process of fungal hyphae and other eukaryotic walled cells has been widely explored, following the earlier indications of their importance by Jaffe, Steer, and their colleagues. Analysis of the literature on fungi, with selected comparison with other tip-growing plant cells, shows that the growth rate and morphology of hyphae are sensitive to factors which influence intracellular Ca^{2+}. These factors include variations in extracellular Ca^{2+} concentrations, Ca^{2+} ionophores, inhibitors of Ca^{2+} transport, and calmodulin- and Ca^{2+}-binding dyes and buffers introduced into the cytoplasm. The effects of these agents appear to be mediated by a tip-high gradient of cytoplasmic free Ca^{2+} which is obligatorily present in all critically examined growing tips. Most recent observations agree that the gradient is very steep, declining rapidly within 10 to 20 μm of the tip. This gradient seems to be generated by the combined effects of an influx of Ca^{2+}, via plasma

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membrane, possibly stretch-activated, channels localized in the hyphal tip, and subapical expulsion or sequestration of these ions. Expulsion probably involves a plasma membrane Ca^{2+}-ATPase, but it is not yet possible to differentiate among mitochondria, endoplasmic reticulum, or vacuoles as the dominant sites of sequestration. It is suggested that regulation of the Ca^{2+} gradient in turn modulates the properties of the actin-based component of the cytoskeleton, which then controls the extensibility, and, possibly, the synthesis of the hyphal apex. Regulatory feedback mechanisms intrinsic to this model of tip growth regulation are briefly discussed, together with suggestions for future experiments which are crucial to its further elucidation and establishment.

Summary: Growth and proliferation of microorganisms such as the yeast Saccharomyces cerevisiae are controlled in part by the availability of nutrients. When proliferating yeast cells exhaust available nutrients, they enter a stationary phase characterized by cell cycle arrest and specific physiological, biochemical, and morphological changes. These changes include thickening of the cell wall, accumulation of reserve carbohydrates, and acquisition of thermotolerance. Recent characterization of mutant cells that are conditionally defective only for the resumption of proliferation from stationary phase provides evidence that stationary phase is a unique developmental state. Strains with mutations affecting entry into and survival during stationary phase have also been isolated, and the mutations have been shown to affect at least seven different cellular processes: (i) signal transduction, (ii) protein synthesis, (iii) protein N-terminal acetylation, (iv) protein turnover, (v) protein secretion, (vi) membrane biosynthesis, and (vii) cell polarity. The exact nature of the relationship between these processes and survival during stationary phase remains to be elucidated. We propose that cell cycle arrest coordinated with the ability to remain viable in the absence of additional nutrients provides a good operational definition of starvation-induced stationary phase.

Summary: Heat shock proteins (Hsps) were first identified as proteins whose synthesis was enhanced by stresses such as an increase in temperature. Recently, several of the major Hsps have been shown to be intimately involved in protein biogenesis through a direct interaction with a wide variety of proteins. As a reflection of this role, these Hsps have been referred to as molecular chaperones. Hsp70s interact with incompletely folded proteins, such as nascent chains on ribosomes and proteins in the process of translocation from the cytosol into mitochondria and the endoplasmic reticulum. Hsp60 also binds to unfolded proteins, preventing aggregation and facilitating protein folding. Although less well defined, other Hsps such as Hsp90 also play important roles in modulating the activity of a number of proteins. The function of the proteolytic system is intertwined with that of molecular chaperones. Several components of this system, encoded by heat-inducible genes, are responsible for the degradation of abnormal or misfolded proteins. The budding yeast Saccharomyces cerevisiae has proven very useful in the analysis of the role of molecular chaperones in protein maturation, translocation, and degradation. In this review, results of experiments are discussed within the context of experiments with other organisms in an attempt to describe the current state of understanding of these ubiquitous and important proteins.

Summary: Micrasterias species have been the subject of numerous experimental studies on cell shape formation in the last 40 years. Chemical and physical treatment during different developmental stages, as well as investigations of ultrastructure by

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means of various different preparation methods, have yielded information about some principles of morphogenesis in the symmetric, highly ornamented Micrasterias cell. The basic symmetry of a Micrasterias cell is determined prior to mitosis and is established without nuclear control thereafter. Normal cell development, however, may occur only under the conditions of continuous protein synthesis throughout the cell cycle. A prepattern for the later cell shape seems to be present at the plasma membrane at the early stages of septum formation. It is realized by a local, patterned distributed incorporation of cell wall material that is delivered by Golgi-produced vesicles. The areas where fusions take place between the primary wall material containing vesicles and the plasma membrane are defined by inward ionic currents that are carried at least in part by calcium. These areas develop into lobes during the following course of cell growth. Cell shaping in Micrasterias cells is thus mediated by both an enhanced extension of the cell wall and an additional incorporation of wall material in the areas of the lobes. Numerous studies have indicated that actin plays an important role in morphogenesis, whereas microtubules do not participate in this process but are involved mainly in nuclear migration. The present review shows that although a wealth of details concerning Micrasterias morphogenesis has already been elucidated, two main questions, i.e., the method of septum formation and the splitting of the lobes, remain to be answered.

Summary: Our understanding of the evolution of DNA restriction and modification systems, the control of the expression of the structural genes for the enzymes, and the importance of DNA restriction in the cellular economy has advanced by leaps and bounds in recent years. This review documents these advances for the three major classes of classical restriction and modification systems, describes the discovery of a new class of restriction systems that specifically cut DNA carrying the modification signature of foreign cells, and deals with the mechanisms developed by phages to avoid the restriction systems of their hosts.

Summary: Anaerobic bacteria include diverse species that can grow at environmental extremes of temperature, pH, salinity, substrate toxicity, or available free energy. The first evolved archaebacterial and eubacterial species appear to have been anaerobes adapted to high temperatures. Thermoanaerobes and their stable enzymes have served as model systems for basic and applied studies of microbial cellulose and starch degradation, methanogenesis, ethanologenesis, acetogenesis, autotrophic CO_2 fixation, saccharidases, hydrogenases, and alcohol dehydrogenases. Anaerobes, unlike aerobes, appear to have evolved more energy-conserving mechanisms for physiological adaptation to environmental stresses such as novel enzyme activities and stabilities and novel membrane lipid compositions and functions. Anaerobic syntrophs do not have similar aerobic bacterial counterparts. The metabolic end products of syntrophs are potent thermodynamic inhibitors of energy conservation mechanisms, and they require coordinated consumption by a second partner organism for species growth. Anaerobes adapted to environmental stresses and their enzymes have biotechnological applications in organic waste treatment systems and chemical and fuel production systems based on biomass-derived substrates or syngas. These kinds of anaerobes have only recently been examined by biologists, and considerably more study is required before they are fully appreciated by science and technology.